

Research Article

Cryptocephaline Egg Case Provides Incomplete Protection from Generalist Predators (Coleoptera: Chrysomelidae)

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The egg case of *Cryptocephalus rufipes* (Goeze) is described and illustrated. In laboratory trials, eggs of field-collected *C. rufipes* were observed for larval emergence (untreated control) or exposed to two species of generalist predators, *Chrysoperla carnea* (Stephens) or *Xylocoris flavipes* (Reuter) in no-choice experiments. The behaviour of the predators upon contact with the *C. rufipes* eggs was observed. The number of hatching larvae was counted and compared. In the presence of each of the two species of predators, larval emergence was significantly reduced. Eggs that were not protected by an egg case were completely consumed by the predators. *C. rufipes* eggs were therefore incompletely protected from the studied generalist predators. This is the first study showing experimentally the protective function of cryptocephaline egg case.

1. Introduction

Leaf beetles in the subfamilies Cryptocephalinae and Lamprosomatinae cover their eggs with small faecal plates. These faecal plates compose a solid egg case that is completely covering the egg. After hatching, the larva is biting a hole in the egg case, and it is wearing it as a larval case. This larval case is continuously enlarged with larval faeces when the larva is moulting and growing [1–3]. One of the functions of the egg and larval cases is thought to be protection from natural enemies [4, 5]. However, both mammal and insect predators and hymenopterous parasitoids are known to accept cryptocephaline larvae as prey or host, respectively [1, 4–9]. First-instar larvae that are still in their egg cases, but not eggs of *Clytra laeviuscula* and *C. quadripunctata*, were observed to be picked up and transported by ants [10]. However, the larvae of these *Clytra* species are known to live inside the nests of ants [11, 12]; consequently the ants are not expected to prey upon the eggs. While some information on natural enemies of larvae accumulated, nothing seems to be known about natural enemies of cryptocephaline eggs. One of the reasons of this gap in knowledge may be the difficulty to find eggs in nature. Some species attach their egg with the help of a stalk to the host plant [13, 14], where they can be observed in the field. However, the eggs of most species drop

to the ground after the female finished building the egg case [3], and then the eggs are difficult to find and observe among the leaf litter. To overcome this problem, in this study adult *Cryptocephalus* (*Burlinius*) *rufipes* (Goeze, 1777) were held in a rearing container, the eggs collected and exposed to two different species of laboratory-reared generalist predators, the common green lacewing *Chrysoperla carnea* (Stephens, 1836) (Neuroptera, Chrysopidae) and the warehouse pirate bug *Xylocoris flavipes* (Reuter, 1875) (Heteroptera, Anthocoridae). Moreover, the egg cases of *C. rufipes* are redescribed and illustrated. *C. rufipes* is widely distributed in Europe, from Portugal to Turkey, and in Northern Africa [15]. In urban areas, it was found feeding on its host plant *Salix purpurea* Linnaeus, 1753, which is planted as a park tree or to form hedges.

2. Materials and Methods

Adult males and females of *C. rufipes* were collected from *Salix purpurea* in Berlin, Friedrichshain (52°52.3272'N, 13°46.5825'E), in June and July, 2014. The adults were kept in 250 mL glass-jars covered with pieces of clothing held with rubber bands at 23 ± 2°C and 60 ± 5% RH. The bottom of the jar was lined with filter paper. A twig of the host



FIGURE 1: Egg of *Cryptocephalus rufipes* (Goeze, 1777), scale = 0.5 mm.

plant *S. purpurea* was placed in a narrow plastic tube (5 × 1.2 cm) filled with water and closed with a plug of paper towel to prevent the water from loss by leakage. The twig was replaced when necessary. Eggs laid by several females were collected daily from the bottom of the jars. The eggs were transferred to Petri-dishes (diameter: 5 cm) lined with paper. To each Petri-dish, five *C. rufipes* eggs and either two adult *X. flavipes*, two larvae of *C. carnea*, or no predators (untreated control) were added. Each treatment had 11 replications. The behaviour of the predators upon first contact with the *C. rufipes*-eggs was observed. In the first three days after adding the predators, the experiments were controlled for survival of the predators. After seven days, eggs were controlled daily for larval emergence. Additionally, five Petri-dishes were prepared with five eggs of *Ephestia kuehniella* each and either two adult *X. flavipes*, two larvae of *C. carnea*, or no predators (untreated control). Laboratory-reared larvae of *C. carnea* and adults of *X. flavipes* were obtained from Biologische Beratung Ltd., Berlin.

The results were analysed with the help of SigmaStat 3.1 software. The number of leaf beetle larvae emerged was subjected to a Kruskal-Wallis One Way Analysis of Variance on Ranks followed by All Pairwise Multiple Comparison Procedures, Dunn's Method, to separate means. Treatments were considered significantly different at the $P = 0.05$ level. Percentage natural mortality data of *C. rufipes* eggs were not corrected for control mortality, because mortality in the untreated control treatment was <5%. The size of 30 eggs, that is, length and width in lateral view, was measured with a measuring ocular mounted on a dissecting microscope.

3. Results

3.1. Field Observations. Beside *C. rufipes*, several other Chrysomelidae were occurring on *Salix purpurea* in June and July, namely *Cryptocephalus (Burlinius) ocellatus ocellatus* Drapiez, 1819, *C. androgyne* Marseul, 1857, *Phratora vitellinae* (Linnaeus, 1758), and *Clytra laeviuscula* (Ratzeburg, 1837). Moreover, the weevil *Polydrusus (Polydrusus) picus* (Fabricius, 1792) was found feeding and mating on *S. purpurea*.

3.2. Morphology of *C. rufipes* Eggs. The eggs of *C. rufipes* are blackish to greyish brown with eight narrow, regular ridges as illustrated in Figure 1. Each individual faecal plate



FIGURE 2: Larva of *Chrysoperla carnea* (Stephens, 1836) uplifting an egg of *Cryptocephalus rufipes* (Goeze, 1777).



FIGURE 3: Adult of *Xylocoris flavipes* (Reuter, 1875) handling an egg of *Cryptocephalus rufipes* (Goeze, 1777).

bears a little crest; the regular arrangement of the faecal plates composes the ridges that have little gaps in case the faecal plates do not perfectly touch. The mean size \pm SD was 0.797 mm \pm 0.057 mm length and 0.532 mm \pm 0.033 mm width; median was 0.80 for length and 0.53 for width. Length was ranging from 0.700 to 0.975 mm, width from 0.475 to 0.600 mm. The eggs are elongate oval; the mean length to width ratio was 1.50 mm \pm 0.095, ranging from 1.25 to 1.70 (median 1.50).

3.3. Experiments with Eggs of *E. kuehniella*. The experiments with eggs of *E. kuehniella* resulted in complete predation of these eggs by both *C. carnea* and *X. flavipes*. In the untreated control, 92% of the *E. kuehniella*-eggs emerged.

3.4. Experiments with Eggs of *C. rufipes*, Behavioural Observations. When encountering the *C. rufipes* eggs, the larvae of *C. carnea* showed the typical prey uplifting behaviour. They fixed the *C. rufipes* eggs and held them in position (Figure 2). *X. flavipes* examined the eggs after contacting it (Figure 3). Within the first three days of the experiment, in all trials one predator consumed the second; consequently, cannibalism occurred.

3.5. Experiments with Eggs of *C. rufipes*, Larval Hatch. Larvae hatched after 10 to 13 days. The presence of the predators significantly affected the number of hatching *C. rufipes*-larvae from the eggs (Kruskal-Wallis One Way Analysis of Variance on Ranks, $H = 18.473$, $DF = 2$, $P < 0.001$). In the untreated control, a mean \pm SD of 3.23 \pm 1.02 larvae hatched. The presence of both *C. carnea* and *X. flavipes* significantly reduced the number of hatching *C. rufipes*-eggs (All Pairwise

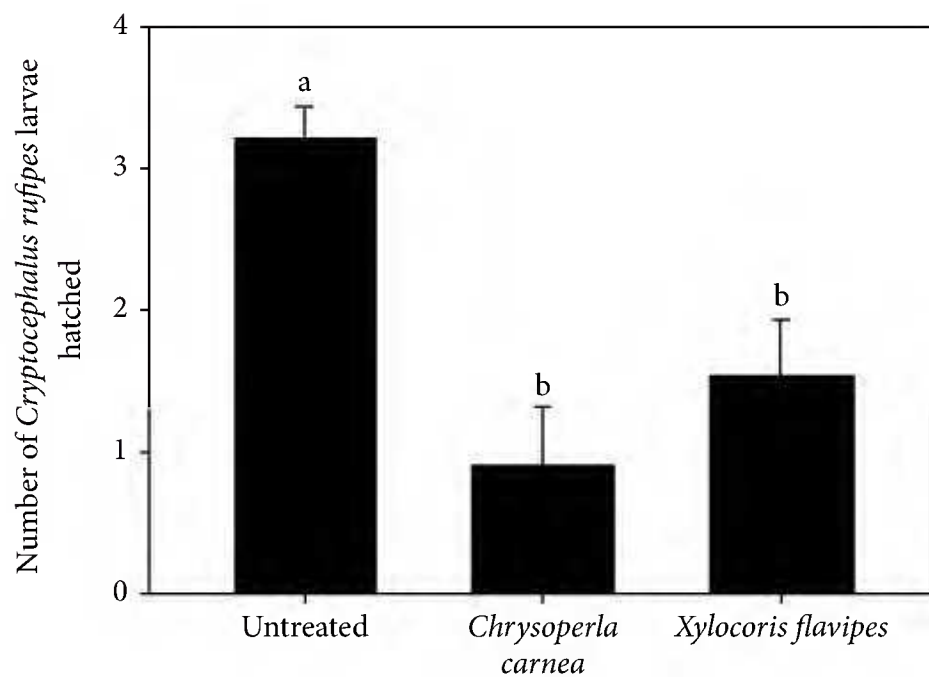


FIGURE 4: Number of *Cryptocephalus rufipes*-larvae hatched out of five eggs exposed to *Chrysoperla carnea* or *Xylocoris flavipes*, or no predators (untreated). Means followed by the same lowercase letter do not differ significantly at $P < 0.05$ (Dunn's Method) ($n = 11$).

Multiple Comparison Procedures, Dunn's Method, $Q = 3.891$ and 2.817 , $P < 0.05$). A mean of 0.91 ± 1.38 and 1.55 ± 1.29 hatched in the presence of *C. carnea* and *X. flavipes*, respectively. However, there was no statistical difference in reduction between the two predators (Dunn's Method, $Q = 0.929$, $P > 0.05$) (Figure 4).

4. Discussion

The egg of *C. rufipes* was first described in 1852 [1], under two synonyms, that is, *C. gracilis* Fabricius, 1792, and *C. minutus* Fabricius, 1792. An egg length of 0.75 mm as well as a surface with eight to nine ridges was given. For *C. gracilis*, regular ridges and a blackish green colour were described, and for *C. minutus* irregular ridges and a yellowish green colour. The egg of *C. minutus* was figured [1, Figure 18]: the egg in this figure is less elongate and the ridges are wider compared to the eggs described in the present study. In 1899, again the egg case was described twice under *C. gracilis* and *C. minutus*, possibly mixing information from [1] and own observations [16]. For the eggs described under *C. minutus* [16, page 51], a length ranging from 0.7 to 0.8 was given, data in accordance with those found in the present study. However, the eggs were described as yellowish grey with slightly irregular ridges, contrary to the blackish brown eggs with rather regular ridges described here. For the eggs described under *C. gracilis* [16, page 56], a short note was given stating a length of 0.8 mm, a surface with nine narrow bend carinae and a greyish colour, fitting better to the eggs described here. However, as the determination of species in the subgenus *Burlinius* Lopatin, 1965, requires in many cases the study of aedeagus characters [17], the identity of immatures described in the 19th Century remains sometimes doubtful, and especially the immatures described as *C. minutus* probably belong to another species. Even though the shape of the eggs is variable as indicated by the range of the length to width ratio, they can be, for example, easily distinguished from those of the related

synoekous species *C. ocellatus*, which are yellowish brown with blunt ridges.

Concerning natural enemies of *C. rufipes*, reports are available about larval parasitoids [1, 16]. No information was traced on natural enemies of eggs of Cryptocephalinae in general.

In the laboratory trials, feeding could not be directly observed, as both species of predators have piercing-sucking mouth parts. However, the behavioural observations indicated the predators identified the *C. rufipes* eggs as prey items. The trials with the *E. kuehniella* eggs showed the ability of the predators to locate the eggs within the experimental arena, and that food was a limiting factor for survival in these no-choice experiments. At least after cannibalism, the predators relied upon the eggs of *C. rufipes* for survival. The analysis of *C. rufipes* hatching showed a significant reduction of larval emergence in the presence of both predators, proving indirectly the predator-induced mortality. However, contrary to the experiments with the eggs of *E. kuehniella*, predation of *C. rufipes* eggs was not complete. This observation suggests egg cases of *C. rufipes* provide a protection against predation by generalist predators, although incomplete.

Larvae of *C. carnea* are known to prey on eggs of various insects [18]. Both adults and larvae of *X. flavipes* are known to prey on insect eggs, including eggs of Chrysomelidae [19]. Both *C. carnea* and related species and anthocorid predators commonly occur in Central Europe. However, they may not frequently encounter *C. rufipes* eggs in the leaf litter because they are typically foraging on leaves. *C. carnea* and *X. flavipes* were used here as model organisms for generalist predators with piercing-sucking mouth parts. Possibly the egg cases provide even better protection against small predators with chewing mouth parts. Natural enemies of cryptocephaline eggs in biotic communities have to be identified and studied in future field studies.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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